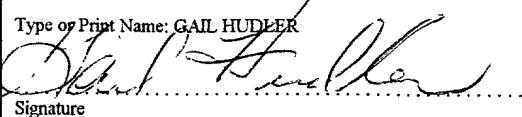
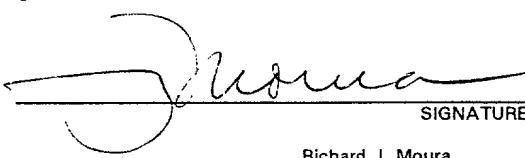


FORM PTO-1390 (REV. 5-93) TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE  ATTORNEY'S DOCKET NUMBER 45687-00008  U.S. APPLICATION NO. 09/462394 (All known to 37 CFR 1.10)
INTERNATIONAL APPLICATION NO. PCT/SE98/01352	INTERNATIONAL FILING DATE 8 July 1998	PRIORITY DATE CLAIMED 9 July 1997
TITLE OF INVENTION REGENERATION OF BIOSENSORS		
APPLICANT(S) FOR DO/EO/US THOMAS CARLSSON		
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:		
<p>1. <input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.</p> <p>2. <input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.</p> <p>3. <input checked="" type="checkbox"/> This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).</p> <p>4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.</p> <p>5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2))</p> <p>a. <input checked="" type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau). b. <input checked="" type="checkbox"/> has been transmitted by the International Bureau. c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US)</p> <p>6. <input type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)).</p> <p>7. <input type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))</p> <p>a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau). b. <input type="checkbox"/> have been transmitted by the International Bureau. c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. d. <input type="checkbox"/> have not been made and will not be made.</p> <p>8. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</p> <p>9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)) (<u>unsigned</u>)</p> <p>10. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</p>		
Items 11. to 16. below concern other document(s) or information included:		
<p>11. <input checked="" type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98</p> <p>12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</p> <p>13. <input checked="" type="checkbox"/> A <b>FIRST</b> preliminary amendment. <input type="checkbox"/> A <b>SECOND</b> or <b>SUBSEQUENT</b> preliminary amendment.</p> <p>14. <input type="checkbox"/> A substitute specification.</p> <p>15. <input type="checkbox"/> A change of power of attorney and/or address letter.</p> <p>16. <input checked="" type="checkbox"/> Other items or information: COPY OF THE INTERNATIONAL SEARCH REPORT PREPARED BY THE EPO; COPY OF NOTICE UNDER PCT RULE 47.1(C); INTERNATIONAL PRELIMINARY EXAMINATION REPORT; AND CONFIRMATION POSTCARD.</p>		

CERTIFICATE OF MAILING BY EXPRESS MAIL	
<p>"EXPRESS MAIL" Mailing Label No. EL269314636US</p> <p>Date of Deposit: <u>16/01/00</u></p> <p>I hereby certify that this paper or fee is being deposited with the U.S. Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231</p> <p>Type of Print Name: GAIL HUDDER</p> <p> Signature</p>	

U.S. APPLICATION NO. (if known see 37 CFR 1.50) <b>09/462394</b>		INTERNATIONAL APPLICATION NO. PCT/SE98/01352		ATTORNEY'S DOCKET NUMBER 45687-00008	
17. <input checked="" type="checkbox"/> The following fees are submitted:				CALCULATIONS	PTO USE ONLY
<b>Basic National Fee (37 CFR 1.492(a)(1)-(5)):</b> Search Report has been prepared by the EPO or JPO ..... \$850.00  International preliminary examination fee paid to USPTO (37 CFR 1.482) \$660.00  No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) ..... \$730.00  Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... \$970.00  International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) ..... \$92.00					
<b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b>				\$ 970	
Surcharge of \$130.00 for furnishing the oath or declaration later than <u>20</u> <u>30</u> months from the earliest claimed priority date (37 CFR 1.492(e)).					
Claims	Number Filed	Number Extra	Rate		
Total Claims	18 - 20 =	0	x \$18.00	\$	
Independent Claims	2 - 3 =	0	x \$78.00	\$	
Multiple dependent claims(s) (if applicable)			+ \$260.00	\$	
<b>TOTAL OF ABOVE CALCULATIONS =</b>				\$ 970	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).					
<b>SUBTOTAL =</b>				\$	
Processing fee of \$130.00 for furnishing the English translation later the <u>20</u> <u>30</u> months from the earliest claimed priority date (37 CFR 1.492(f)).					
<b>TOTAL NATIONAL FEE =</b>				\$ 970	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property					
<b>TOTAL FEES ENCLOSED =</b>				\$ 970	
				Amount to be: refunded	\$
				charged	\$
a. <input checked="" type="checkbox"/>	Check in the amount of \$ <u>970.00</u> to cover the above fees is enclosed.				
b. <input type="checkbox"/>	Please charge my Deposit Account No. <u>10-0447</u> in the amount of \$ <u>          </u> to cover the above fees. A duplicate copy of this sheet is enclosed.				
c. <input checked="" type="checkbox"/>	The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>10-0447</u> . A duplicate copy of this sheet is enclosed.				
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.					
SEND ALL CORRESPONDENCE TO:					
Richard J. Moura, Esq. Jenkens & Gilchrist, P.C. 3200 Fountain Place 1445 Ross Avenue Dallas, Texas 75202-2799 214/855-4500					
 SIGNATURE <u>Richard J. Moura</u> NAME					
<u>34,883</u> REGISTRATION NUMBER					

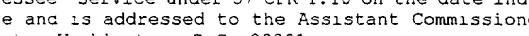
09/462394  
514 Rec'd PCT/PTO 06 JAN 2000

Patent Application  
Docket No.: 45687-00008

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: §  
Thomas Carlsson §  
Serial No.: Not Assigned § Art Unit: Not Assigned  
Filed: Herewith § Examiner: Not Assigned  
For: REGENERATION OF BIOSENSORS

BOX PATENT APPLICATION  
Assistant Commissioner for  
Patents  
Washington, D.C. 20231

CERTIFICATE OF MAILING BY EXPRESS MAIL	
"EXPRESS MAIL" Mailing Label No. EL269314636US	
Date of Deposit: January <u>6</u> , 2000 . . . . .	
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Type or Print Name	GAIL HUDLER
 Signature	

Sir:

**PRELIMINARY AMENDMENT**

Prior to commencing the examination of the above-identified application, please amend the above-identified application as set forth below to place the application in condition for allowance.

**In the Claims:**

Please amend Claims 1, 3, 4, 6-10, and 12-18 as follows:

1           1. (Amended) A method of regenerating a biosensor of the  
2       type having a signal generating portion responsive to some  
3       property of or to the presence of some component in a biological  
4       fluid, and having a flow passage through which fluid is being  
5       passed at selectable flow rates, the method comprising:

6               (a) passing a background flow of fluid without  
7       response generating components through the flow  
8       passage;

9               (b) at a selected point in time introducing a sample  
10      aliquot into said background flow; and

11      [characterized by]

12               (c) increasing the flow rate of the background fluid  
13       at a point in time when at least a fraction of said  
14       sample aliquot has entered said flow passage.

15      3. (Amended) The method of claim 1 [or 2], wherein said  
16      flow rate is increased by 5-100%, preferably 10-50%, most  
17      preferably 15-30%.

18      4. (Amended) The method of claim 1 [or 2], comprising  
19      maintaining the increased flow rate until the signal from the  
20      sensor has reached a preselected value.

1               6. (Amended) The method of [any preceding claim] claim 1,  
2       wherein the increased flow rate is maintained for 10-60 s,  
3       preferably 20-40 s.

1               7. (Amended) The method of [any preceding claim] claim 1,  
2       wherein said background flow is 0.1 - 10 ml/min., preferably  
3       1 ml/min.

1               8. (Amended) The method of [any preceding claim] claim 1,  
2       wherein said increase in flow rate is initiated when the entire  
3       sample has entered said flow passage.

1               9. (Amended) The method of [any preceding claim] claim 1,  
2       wherein sample is continuously drawn from a sample source, and  
3       when not being analyzed it is disposed as waste.

1               10. (Amended) The method of [any preceding claim] claim 1,  
2       wherein the sample is blood, optionally premixed with  
3       anticoagulant.

1               12. (Amended) A system for continuous monitoring of  
2       analytes in a biological fluid, the system having increased life

3 by virtue of inherent regeneration of sensors employed, the  
4 system comprising:

5 (a) a biosensor [(26, 30, 32)] of the type having a  
6 flow passage through which fluid is being passed at  
7 selectable flow rates, and a signal generating portion  
8 located in said flow passage and responsive to some  
9 component or property of a biological fluid;

10 (b) a sampling device [(4)] for providing a sample of  
11 said biological fluid;

12 (c) means [(10, 15, 18, 24)] for passing a flow of a  
13 background fluid through said flow passage at  
14 selectable flow rates;

15 (d) means [(20, 50, 55)] for injecting said sample  
16 into said flow of background fluid at selectable  
17 points in time to provide a combined flow;

18 (e) means [(50, 55)] for increasing the flow rate of  
19 said combined flow at a selectable point in time  
20 during passage of the sample through said flow passage  
21 in order to achieve a washing action on the signal  
22 generating portion; and

23 (f) means [(30, 32)] for providing a signal from said  
24 signal generating portion.

1                   13. (Amended) The system of claim 12, wherein said  
2 sampling device comprises a catheter [(4)] insertable in a blood  
3 vessel of a human or an animal, and tubing [(8)] connecting the  
4 catheter to the system.

1                   14. (Amended) The system of claim 12 [or 13], wherein said  
2 means for passing a flow of a background fluid through said flow  
3 passage at selectable flow rates comprises a pump [(10)] and  
4 appropriate tubing [(18, 24)].

1                   15. (Amended) The system of claim 12[, 13 or 14], wherein  
2 said means for injecting said sample into said flow of  
3 background fluid, comprises a valve [(20)] switchable between  
4 injection and waste disposal modes.

1                   16. (Amended) The system of [any of claims 12-15]  
2 claim 12, wherein said means for increasing the flow rate  
3 comprises a control unit [(50)] programmed to respond to signals  
4 from said sensor.

1                   17. (Amended) The system of [any of claims 12-16]  
2 claim 12, wherein said means for providing a signal from said

3 signal generating portion comprises at least one thermistor  
4 [(30, 32)].

1 18. (Amended) The system of [any of claims 12-17]  
2 claim 12, further comprising a connector [(100)], for connecting  
3 said sampling device [(4)] to said pump [(10)], the connector  
4 comprising:

5 a male [(102)] and a female [(104)] part,  
6 a tube [(108)] of a hard material such as steel having  
an inner diameter, and being inserted in the center of one of  
said male [(102)] and female [(104)] parts and protruding from  
an end surface [(118)] of said part [(104; 102)];

10 a catheter [(106)] of a soft material inserted in the  
center of the other of said male [(102)] and female [(104)]  
parts and having an inner diameter substantially smaller than  
the inner diameter of said tube [(108)], and having an  
14 essentially flat end surface [(126)], wherein

15 the protruding end of said tube is ground such as to  
16 form a sharp circumferential edge [(112)], and wherein

17 the positions of said tube [(108)] and said catheter  
18 [(106)] in their respective male or female part, are such that  
19 when said male and female parts are connected, said sharp edge

20        [(112)] penetrates into said catheter [(106)], thereby forming  
21        a fluid tight connection.

REMARKS

Applicant has amended the claims of the PCT Patent Application No. PCT/SE98/01352 filed on July 8, 1998, from which priority is claimed.

In view of the forgoing amendment, the claims now pending in this application are respectfully submitted to be in condition for allowance, and action to that end is respectfully requested.

Respectfully submitted,

By:   
Richard J. Moura  
Reg. No. 34,883

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**VERIFIED STATEMENT CLAIMING SMALL ENTITY STATUS  
(37 CFR 1.9(f) & 1.27(b)) - INDEPENDENT INVENTOR**

Applicant : Thomas CARLSSON

Serial or Patent No.: Not Yet Assigned

Filed or Issued: January 6, 2000

Title: REGENERATION OF BIOSENSORS

As a below named inventor, I hereby declare that I qualify as an independent inventor as defined in 37 CFR 1.9(c) for the purposes of paying reduced fees to the Patent and Trademark Office with regard to the invention as described in:

the specification filed herewith.  
 application serial no. 09/462,394, filed January 6, 2000.  
 patent no. \_\_\_\_\_, issued \_\_\_\_\_.

I have not assigned, granted, conveyed or licensed and am under no obligation under contract or law to assign, grant, convey or license, any rights in the invention to any person who could not be classified as an independent inventor under 37 CFR 1.9(c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR 1.9(c) or a nonprofit organization under 37 CFR 1.9(c).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

no such person, concern, or organization  
 persons, concerns or organizations listed below\*

\* NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Name of Inventor :

Thomas CARLSSON



Signature of Inventor

Date: 000417

REGENERATION OF BIOSENSORS

The present invention relates in general to the field of biosensors, and in particular to methods and apparatus for regenerating such sensors, thereby increasing the effective life thereof.

In a specific aspect the invention relates to a system for continuous analysis of analytes in blood or serum comprising means for regeneration of the sensor employed therein.

**Background of the Invention**

Measurements of analytes in blood is commonly performed by sampling blood from patients and analyzing said samples in a laboratory, often situated at a location remote from the ward. E.g. for glucose analysis there are available special reagent sticks usable for measuring on site i.e. in the ward. However, the accuracy of such measurements is questionable, and the error could be 10 - 20% at best.

Often it is necessary to perform several sequential measurements over periods of several hours, which is very labor intensive. Furthermore, the risk for errors because of the human intervention is evident, and the low accuracy is of course also a drawback in this regard.

For the purposes of this application, the term "biosensor" means any device having a portion which interacts with biological or biochemical material, and has the capability to generate a signal indicative of a change in some parameter of said biological or biochemical material as a consequence of said interaction.

When analytes such as glucose, urea, lactate, ATP, glycerol, creatinine and pyruvate in biological samples, such as blood, plasma or serum are analyzed using biosensor techniques based on immobilization of enzymes, the sensor surface will be exposed to a certain amount of sample during a certain time sufficient to achieve an adequate sensor response. It is well known that the sensor response gradually will degrade because of fouling of the surface. This in its turn is a consequence of said exposure and the interaction between the surface and the substances present in the sample that occurs. The chemical and physical composition of the sample is thereby of importance, the sample i.a. comprising red cells, blood platelets, macromolecules, electrolytes, lipids, red/ox-compounds etc. It is also known that the support material for the enzyme immobilization in biosensors based on enzyme column technology is fouled by the substances present in the sample.

In cases where selective membranes are used for protection of the sensor surface of biosensors based on enzyme electrode technology, said membranes are also fouled by such substances. This fouling influences the sensor response by substantially reducing the life and stability of the biosensor.

## 25 Description of Related Art

Most known metabolite sensors today are based on the amperometric principle, that is measurement of oxygen consumption or hydrogen peroxide production in electrochemical reactions. However, interference with reducing/oxidizing substances causes problems like long time drift, need for frequent calibration and short life. Regarding the sampling procedure there exist devices which, before the actual

measurement, condition the blood before it enters the actual sensor by e.g. introducing a special step, such as dialysis. This is both a more complicated solution and also more 5 expensive, since the dialysis cassette has to be replaced before a new measurement can be made.

Another known sensor principle is by utilizing the heat production when the analytes are decomposed by the appropriate 10 enzyme for the analyte in question. This so called enzyme calorimeter principle is known from US-4,021,307. The enzyme calorimeter disclosed therein is however not suited for direct measurement on whole blood, since the blood cells quickly will clog the column containing the immobilized enzyme, due to 15 adsorption of blood constituents such as various cells, trombocytes, proteins etc. This effect could be circumvented to a certain extent by diluting the blood at least ten times, which will reduce the sensitivity of the measurement considerably. However such a measure would require an extra 20 supply of a diluting solution. Another way of reducing the clogging of the column is to use a special super porous support material with a pore size larger than 10  $\mu\text{m}$ . This support made from agarose, is however softer than the conventional support materials used in this field, preferably glass, and therefore 25 are at a certain risk of (occasionally) being compressed by the blood sample, which in turn quickly will clog the column.

Thus, at present there is no reliable method and system 30 available for the direct and continuous analysis of whole blood drawn from patients.

**Summary of the Invention**

The present invention therefore seeks to provide an improved method of analyzing whole blood in respect of analytes such as glucose, lactate, urea, ATP, glycerol, creatinine and pyruvate wherein the drawbacks of the prior art methods are alleviated.

In particular the active life of a biosensor that is used for such analysis is prolonged by providing for reduced fouling of the sensor by regenerating the sensor in accordance with the invention.

The method according to the invention is defined in claim 1.

In a second aspect of the invention there is also provided a system for long time measurements of whole blood directly and continuously sampled from a patient, wherein the flow of the sampled blood is held at a very low rate.

The system according to the invention is defined in claim 12.

Further scope of applicability of the present invention will become apparent from the detailed description given hereinafter.

However, it should be understood that the detailed description and specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

The present invention will become more fully understood from the detailed description given hereinbelow and the accompanying

drawings which are given by way of illustration only, and thus not limitative of the present invention, and wherein

5 Fig. 1 is an overview of a system according to the invention;

Fig. 2 is a cross sectional view through a connector according to the invention;

10 Fig. 3 is a view similar to Fig. 2, but showing a conventional connector without the inventive sealing feature; and

15 Fig. 4 is a flow chart illustrating the sequence of steps in the method of the invention.

#### Detailed Description of Preferred Embodiments

20 In Fig. 1 there is disclosed a system for performing a continuous monitoring of the concentration of analytes in blood.

It comprises a blood sampling device 2, such as a cannula 4  
25 inserted in a vein of a patient. The cannula 4 is connected to the other system components via a tubing 8 and a suitable connector 6 (to be described) connecting to a pump 10, which is provided for drawing the various fluids through the system at controlled flow rates. The pump is a multichannel pump, and has  
30 a first input 12 for the blood from the sampling device 2, a second input 14 for buffer solution from a buffer storage 15, and a third input 16 for anticoagulant from an anticoagulant

reservoir 17. Anticoagulant is fed through a line 9 into the sample flow in line 8 at a point near the tip of the catheter 4.

5 Alternatively there may be provided separate pumps for the various components.

The valve 20 is important for the operation in accordance with the invention, and has two inputs, one input 21 for sample 10 blood, fed from pump 10 through line 22, and one input 23 for buffer, fed through line 24. There are also two outputs, a first connecting to line 25a feeding the fluid to the analysis portion, and a second connecting to line 25b for discharging the fluid as waste. The valve 20 is designed such as to permit fluid 15 (i.e. blood in this embodiment) from line 22 to be injected into the buffer flow from line 24.

All surfaces in the system exposed to sample are coated with heparin in order to make the system blood compatible.

20 The actual sample analysis may be carried out in a so called enzyme reactor (ER) 26, although it is contemplated that any biosensor type may be used, provided it has a sensitive portion arranged in some kind of flow passage where flow past the 25 sensitive portion of the sensor can be controlled. Thus, a sensor of a type that is merely immersed in a liquid would not be suitable for use with this invention. An ER is used in a preferred embodiment and will be described in more detail below.

30 The system also comprises a control unit 50, which may be a micro processor or a PC. An interface 55 is connected between the control unit 50 and the components in the system, such that control signals to the pump 10 and the valve 20 are fed via

lines 52 and 54 respectively. Thus, the pumping rate in the various independently operated flow passages may be increased or decreased, and the valve 20 may be switched between its various 5 positions by commands issued by the control unit in response to signals from the biosensor. An amplifier 56 is provided for amplifying the signals from the biosensor 26, and feeding said signals to the interface on line 58, for further transmission to the control unit which uses the information thus obtained to 10 issue the appropriate control commands to the pump and valve.

#### The Enzyme Reactor

An enzyme reactor (ER) 26 comprises a sensor column. The column 15 contains support material such as beads of glass or hard polymer resin, on which enzyme has been immobilized. Immobilization of enzyme is standard procedure and does not form part of this invention, and will hence not be described in detail herein.

20 The operation and function of the ER 26 is as follows.

The sensor column has two thermistors 30, 32, one 30 arranged at the column inlet and the other 32 at the column outlet. Fluid entering the column will begin reacting with the enzyme that is 25 immobilized on the beads in the column, and will thereby generate heat, causing the temperature of the fluid to increase. By monitoring the temperatures at the inlet and outlet respectively, and integrating the temperature over time, the integral obtained will correspond to the heat of reaction, which 30 then may be related to the concentration of e.g. glucose in the fluid.

Because of non-specific reactions that may be exothermic or endothermic and which occur in the column, temperature fluctuation must be accounted for. Thermostating the reactor is 5 one way of doing this, but it can be achieved also in other ways and by other means, and is not crucial to the invention.

Operation

10 Returning now to Fig. 1, there is illustrated an embodiment of the system which comprises a biosensor 26 arranged in a thermostated environment. The system is operated as follows:

15 The catheter 4 is inserted in a blood vessel of a patient, and connected to the tubing of the system by means of a connector 6 (to be described). Initially the pump will draw blood through line 8 via line 22, through the valve 20 and to the waste line 25b for disposal, and buffer from buffer storage 15 is drawn via line 18 through the valve 20 and via line 25a into the enzyme 20 reactor 26. The continuously measured signals obtained from the sensor when the buffer passes through it will form a background or zero level, and the flow of buffer will be referred to as a "background flow" in this application. The rate of background flow may vary in the range 0.1-10 ml/min., and preferably is 1 25 ml/min.

30 If desired, and indeed it is mostly required, anticoagulant is mixed with the blood. Normally a ratio between sample and anticoagulant of 1:1 will be used, although other ratios are conceivable for specific conditions. The anticoagulant is pumped in line 9 and injected in the sample flow line 8 near the catheter 4 tip.

At a time when it is desired to make a measurement the valve is given a "SWITCH TO INJECTION MODE" command to the effect that the blood is redirected into the buffer stream, for a period of 5 time of a duration sufficient for an aliquot of 10  $\mu$ l to be entered as a liquid plug in the buffer stream (other sample volumes may of course be employed, but at the present time 10  $\mu$ l has proven suitable in most cases). This "blood plug" is passed in line 25a, which runs in the thermostated medium, where the 10 sample obtains a controlled temperature, and then it enters the ER 26.

As soon as the blood, containing e.g. glucose, reaches the ER 26, the glucose will start reacting with the enzyme, thereby 15 evolving heat of reaction. The enzyme reaction is a very rapid process. Other components in the blood, such as various cells, trombocytes and proteins having a tendency to adsorb to the material inside the reactor, will begin to adsorb. The latter process is however a slow process compared to the diffusion 20 controlled enzyme reaction. The small glucose molecules diffuse very much faster than the macromolecules and other macro components in the blood.

The thermistor 32 at the output end of the ER 26 will experience 25 a rise in temperature caused by the enzyme reaction occurring in the reactor (at this time the entire sample preferably should have entered the reactor, although this is not absolutely necessary, as will be discussed below).

30 In the present embodiment the temperature increase sensed by thermistor 32 is transmitted to the control unit which is programmed to respond to an increase in the temperature signal to issue a INCREASE BUFFER FLOW RATE command to the pump 10 to

increase the rate of flow of the buffer by 5-100%, preferably by 10-50%, most preferably by 15-30%.

5 By balancing the flow rates, i.e. defining a suitable ratio between background flow rate and increased flow rate, it is possible to create a situation where larger components, such as cells, proteins etc, are washed away before they have had an opportunity to adsorb on the active surfaces inside the ER 26,

10 and at the same time allow the smaller molecules of interest sufficient time to react with the enzyme to such an extent that it is possible to detect the reaction.

15 This balancing of flow ratios within the given limits is made by straight forward routine experimentation for a given system, and is easily done by the skilled man.

20 In an alternative embodiment it may be sufficient if only a fraction of the sample has entered the reactor. In this case the detection of signal onset is not used for triggering. Instead a certain time is determined empirically, namely the time it takes for the sample to just about reach the reactor after injection into the background flow. This time is then programmed into the control unit and used as a starting point for increased flow.

25 This time can of course be selected such that different fractions of sample enter the reactor. It should be noted that if only a very minor fraction has entered when increased flow is initiated, the signal will be low; however, in most cases the entire sample will have reached the reactor by virtue of the

30 void volume of the reactor being substantially larger than the sample volume.

It could also be possible to wait a short time, such as up to 5 seconds, after the sample completely has entered the reactor, i.e. after the detection by thermistor 32, before increasing the 5 flow. Thus, in fact there is a time interval during which increased flow can be performed. The actual set of parameters has to be found empirically for each individual system, and the skilled man will be able to find these parameters without inventive work.

10

The flow pulse at the higher flow rate is maintained until a preselected signal value from the reaction response has been recorded, e.g. a peak maximum, and at this point the flow will be decreased by a RETURN TO NORMAL FLOW command to the pump 10, 15 thereby stopping the additional flow of buffer solution. The duration of the pulse of increased flow rate may be 10-60 s, preferably 20-40 s.

Temperature fluctuations may be eliminated by thermostating the 20 system, e.g. having the ER 26 immersed in a controlled temperature bath.

Fig. 4 illustrates the control algorithm in a simplified flow chart form.

25

When it is desired to make a measurement, the operator may select a INJECT command from a menu, or the computer may be programmed to issue the command at a preselected point in time. This command will set the valve such that the flow of sample 30 (blood) is diverted into the buffer flow, for a time sufficient to inject the desired sample volume, i.e. 10  $\mu$ l. Then, the valve is reset to normal mode, i.e. the blood is discharged as waste.

If the system parameters, such as configurations, flows etc. are well defined, then it is possible to preset the time  $T$  when increased flow is to be initiated. Thus, when the elapsed time  $t$  after injection of sample equals  $T$ , increasing is initiated by the computer.

Alternatively, the computer continuously registers the signal from the thermistor, and when a signal gradient, i.e. a temperature rise, of sufficient magnitude occurs, increasing the flow is initiated.

The increase in flow is performed by increasing the pump speed, by the computer issuing a INCREASE PUMP SPEED command to the interface, such the initial pump rate  $P_0$  is increased by a factor corresponding to an increase of 5-100%, preferably by 10-50%, most preferably by 15-30%. Thus, the pump speed during increased flow (or pulse) mode is

$$P = P_0 + X P_0.$$

20

After a time  $\Delta t$  when the reaction in the reactor is complete, the pump speed is reverted back to the initial value  $P_0$ .

Then, control reverts to the computer for either a programmed new measurement at a preselected point in time, or an operator initiated measurement.

#### The Connector

30 In fig. 2 a connector device for connecting to a patient, suitable for use in connection with the system according to the invention is illustrated.

The connector device, generally designated 100, comprises a male part, generally designated 102, and a female part, generally designated 104.

5

The male part 102 is provided on the distal end of a catheter 106 that has been inserted in e.g. a blood vessel of a patient.

The female part 104 comprises a narrow tube 108 of e.g. steel, 10 the inner diameter of which is larger than the inner diameter of the lumen 110 of the catheter 106. The ratio between diameters is preferably 2-3:1. Furthermore, the steel tube 108 is milled or ground on its outer proximal end such that a sharp cutting edge 112 is formed, i.e. the outer surface is made slightly 15 conical at the proximal end.

The tube 108 is inserted in and fixed centrally of a concentric socket structure 114, forming said female part 104. The socket 114 thus comprises a cylinderlike element having a 20 circular/cylindrical opening or bore 116, the inner surface of which is slightly tapered, and in the center of which the tube 108 protrudes a fractional distance of the depth of said opening. Thus the cutting edge 112 of the tube 108 is located 25 somewhere in the region between the bottom 118 of said opening 116 and its peripheral edge 120. The tapering is such that the diameter at the bottom 118 is slightly smaller than the diameter at the peripheral edge 120.

Similarly, the catheter is located centrally of a cylindrical 30 member 122 forming the male part 102, the outer diameter of which snugly fits inside the opening of the female part 104. The end surface 124 of the catheter 106 is flush with the end

surface 126 of the cylindrical member 122. The tube 108 extends so much away from the bottom 118 of the female part that when the male and female parts are connected, the sharp edge 112 will 5 penetrate the end surface of the catheter 106.

The catheter 106 is made of a material that is enough resilient or soft, that when the male and female parts are connected, the cutting edge 112 sinks into the end surface 124 of the catheter 10 106. Thereby a reliable and safe connection is provided in the transport of blood from the patient to the measuring system. Suitable materials for the catheter are e.g. soft PVC/silicone.

An example of a suitable locking device usable with the 15 connector and having a male/female structure as outlined above is a Luer®-type lock.

As can be seen in the figure there is an abrupt change in the 20 flow cross-section at the connection between catheter 106 and tube 108. This is essential in the sense that it will prevent or alleviate clogging of the flow path. This principle is known.

By providing the direct contact connection between tube 108 and catheter 106, as disclosed above, the large volume 116 is 25 eliminated from the flow path. This is important in the sense that if the blood would have to pass such a large volume before entering the tube 108, there would be enough time for the constituents of the blood to adhere to the inner surfaces of said volume 116. In Fig. 3 a connector comprising an ordinary 30 Luer-lock type coupling is shown. The male part 102 and the female part 104 are connected such as to form a dead space 119. It is self evident that the flow rate will decrease drastically

when the blood enters the large dead space 119 inside the coupling, thereby giving the blood constituents time to adhere to the inner surface of the connector and eventually clog the 5 connector.

Of course it is equally conceivable to provide the catheter in the female part and the tube in the male part. However, the first embodiment is preferred since the sharp edge of the tube 10 will be protected if arranged "inside" the female part, as shown.

Other types of couplings are of course conceivable, the 15 important feature is the provision of a sharp edge on the tube, and a catheter having the necessary softness or resiliency that the edge will actually sink into the material when the parts are connected.

For example one could envisage some type of screw and nut 20 connector, or a bayonet type coupling.

The invention will now be further illustrated by way of the following non-limiting Examples.

25 EXAMPLES

The following Examples were performed with the setup shown in Fig. 1. The sensor was an enzyme reactor having dimensions 20 mm length x 4 mm diameter.

30

The skilled man will easily be able to select suitable thermistors having the appropriate properties. One example of thermistor is obtained from Victory Eng. Inc.

The sample volume was 5 or 10  $\mu$ l, and the background flow was 1 ml/min.

5 Signal values are given in Volts.

Example I (comparative)

In this example the flow was kept constant, and thus no

10 increased flow was applied. The sample (blood) volume was 5  $\mu$ l, and the base line signal was recorded before and after detection was made. Three consecutive runs were performed.

<u>Signal/V</u>			
15	<u>Sample No.</u>	<u>Before det.</u>	<u>After det.</u>
	1	0,10	0,15
	2	0,15	0,23
	3	0,22	0,34

20 As is clearly demonstrated the baseline signal before detection increases from 0,10 to 0,22 V, and also the baseline signal after detection increases from 0,15 to 0,34 V.

Example II (comparative)

25

The experiment of Example I was repeated with a fresh sensor and new samples.

	<u>Sample No.</u>	<u>Before det.</u>	<u>After det.</u>
30	4	0,14	0,38
	5	0,35	0,63

Again the base line signals clearly are not reproducible between runs.

5 Example III (comparative)

In this example the flow was also kept constant but a sample prepared from a standard solution and glucose was introduced, and passed through the reactor.

10

<u>Sample No.</u>	<u>Before det.</u>	<u>After det.</u>
6	0,30	0,31
7	0,29	0,31

15 As can be seen, the base line signal is not affected.

Example IV (comparative)

The same conditions as in Example I, but the sample volume is  
20 increased to 10  $\mu$ l.

<u>Sample No.</u>	<u>Before det.</u>	<u>After det.</u>
8	0,55	0,64
9	0,59	0,67
25 10	0,62	0,70

Again, the base line is not reproducible between runs.

Example V (according to the invention)

30

In this example the sample volume was 10  $\mu$ l. When the onset of sensor response was detected, the buffer flow was increased by

15% and maintained at that level for 20 seconds, when the response signal began to decrease again.

	<u>Sample No.</u>	<u>Before det.</u>	<u>After det.</u>
5	11	0,23	0,22
	12	0,23	0,22
	13	0,23	0,22
	14	0,22	0,22
10	15	0,21	0,22
	16	0,20	0,22
	17	0,21	0,22
	18	0,21	0,21
	19	0,23	0,21

15

As can be seen from the table, 9 consecutive runs were made and the base line returned reproducibly to the same level within the accuracy that measurements allow.

20 The system described herein is preferably designed as a "bed-side monitor", i.e. a portable system for making around-the-clock surveillance of e.g. intensive care patients, or patients undergoing dialysis.

25 The control unit and other hardware components are thereby integrated in one single piece of equipment that is easily moved from one location to another.

30 Although the description has been made with reference to a system and method for analyzing analytes in blood, it is equally possible to use the inventive ideas for other types of complex biological/biochemical media, such as fermentation media, animal cell culture media.

For example it is suitable for analyzing ethanol or residual sugar in mash in brewing processes. It could also be used for analyzing various substances, e.g. insulin, amino acids or

5 growth hormone in cell culture media.

It could also be used to analyze various components in milk or similar foodstuffs.

10 The skilled man could envisage numerous other applications of the basic principle of the invention, and implement them without inventive work.

Such variations are not to be regarded as a departure from the  
15 spirit and scope of the invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

## CLAIMS:

1. A method of regenerating a biosensor of the type having  
5 a signal generating portion responsive to some property of or  
to the presence of some component in a biological fluid, and  
having a flow passage through which fluid is being passed at  
selectable flow rates, the method comprising:

10 a) passing a background flow of fluid without  
response generating components through the flow  
passage;

15 b) at a selected point in time introducing a sample  
aliquot into said background flow;

characterized by

20 c) increasing the flow rate of the background fluid  
at a point in time when at least a fraction of said  
sample aliquot has entered said flow passage.

2. The method of claim 1, comprising detecting the  
presence of the sample by the sensor and increasing the flow  
25 rate at a point in time 0-30 seconds after the presence of  
sample is detected, preferably 0-20 seconds, more preferably 0-  
10 seconds, and most preferably immediately after such  
detection.

30 3. The method of claim 1 or 2, wherein said flow rate is  
increased by 5-100%, preferably 10-50%, most preferably 15-30%.

35 4. The method of claim 1 or 2, comprising maintaining the  
increased flow rate until the signal from the sensor has  
reached a preselected value.

5. The method of claim 4, wherein said preselected value is a signal peak maximum.

5 6. The method of any preceding claim, wherein the increased flow rate is maintained for 10-60 s, preferably 20-40 s.

7. The method of any preceding claim, wherein said 10 background flow is 0.1 - 10 ml/min., preferably 1 ml/min.

8. The method of any preceding claim, wherein said increase in flow rate is initiated when the entire sample has entered said flow passage.

15 9. The method of any preceding claim, wherein sample is continuously drawn from a sample source, and when not being analyzed it is disposed as waste.

20 10. The method of any preceding claim, wherein the sample is blood, optionally premixed with anticoagulant.

11. The method of claim 10 wherein said anticoagulant is premixed with blood in a ratio of 1:1.

25 12. A system for continuous monitoring of analytes in a biological fluid, the system having increased life by virtue of inherent regeneration of sensors employed, the system comprising

30 a) a biosensor (26, 30, 32) of the type having a flow passage through which fluid is being passed at selectable flow rates, and a signal generating portion located in said flow passage and responsive to some component or property of a 35 biological fluid,

b) a sampling device (4) for providing a sample of said biological fluid;

5 c) means (10, 15, 18, 24) for passing a flow of a background fluid through said flow passage at selectable flow rates;

10 d) means (20, 50, 55) for injecting said sample into said flow of background fluid at selectable points in time to provide a combined flow;

15 e) means (50, 55) for increasing the flow rate of said combined flow at a selectable point in time during passage of the sample through said flow passage in order to achieve a washing action on the signal generating portion; and

20 f) means (30, 32) for providing a signal from said signal generating portion.

25 13. The system of claim 12, wherein said sampling device comprises a catheter (4) insertable in a blood vessel of a human or an animal, and tubing (8) connecting the catheter to the system.

30 14. The system of claim 12 or 13, wherein said means for passing a flow of a background fluid through said flow passage at selectable flow rates comprises a pump (10) and appropriate tubing (18, 24).

35 15. The system of claim 12, 13 or 14, wherein said means for injecting said sample into said flow of background fluid, comprises a valve (20) switchable between injection and waste disposal modes.

16. The system of any of claims 12-15, wherein said means for increasing the flow rate comprises a control unit (50) programmed to respond to signals from said sensor.

5

17. The system of any of claims 12-16, wherein said means for providing a signal from said signal generating portion comprises at least one thermistor (30, 32).

10 18. The system of any of claims 12-17, further comprising a connector (100) for connecting said sampling device (4) to said pump (10), the connector comprising

15 a male (102) and a female (104) part,

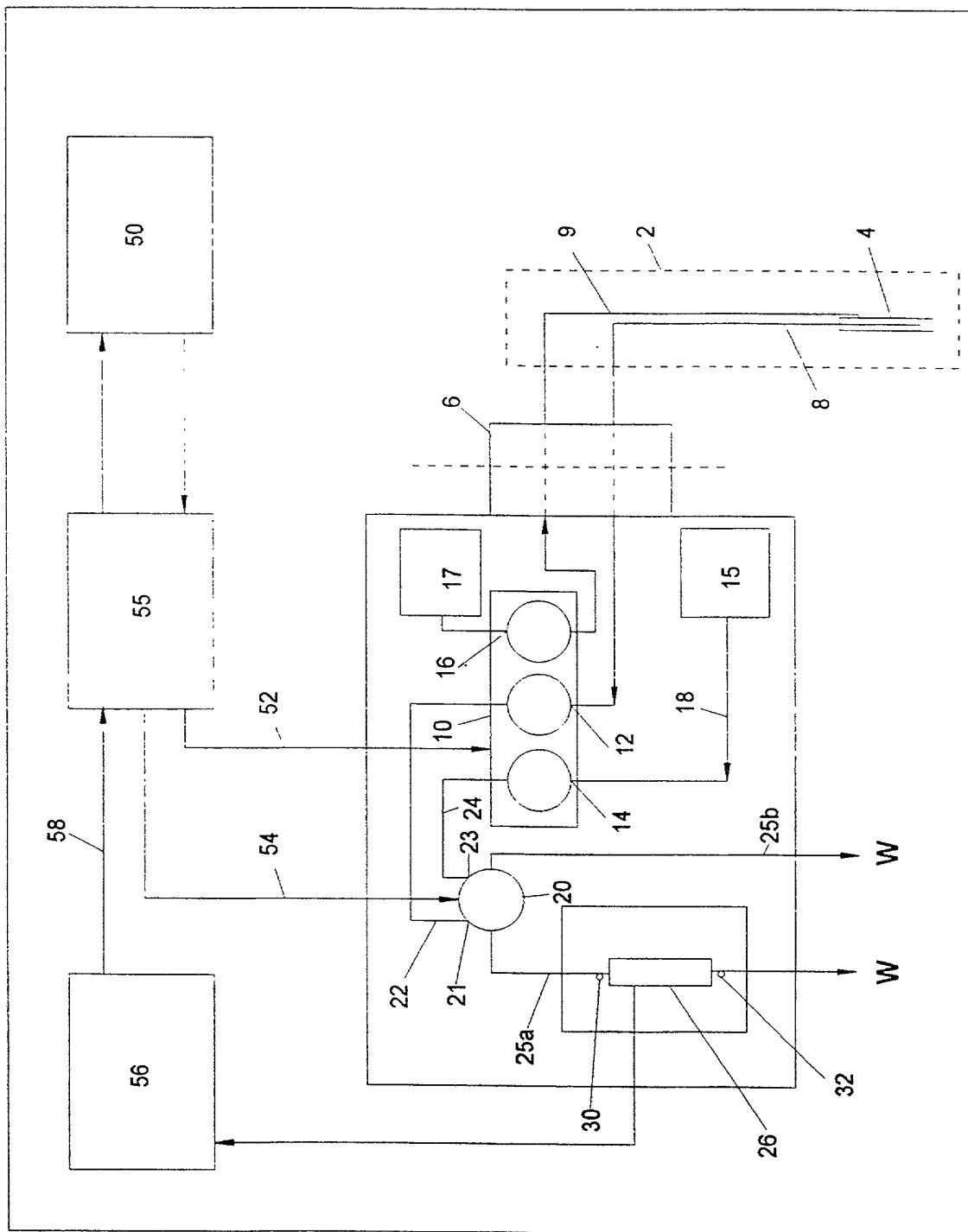
15 a tube (108) of a hard material such as steel having an inner diameter, and being inserted in the center of one of said male (102) and female (104) parts and protruding from an end surface (118) of said part (104; 102),

20 a catheter (106) of a soft material inserted in the center of the other of said male (102) and female (104) parts and having an inner diameter substantially smaller than the inner diameter of said tube (108), and having an essentially 25 flat end surface (126), wherein

the protruding end of said tube is ground such as to form a sharp circumferential edge (112), and wherein

30 the positions of said tube (108) and said catheter (106) in their respective male or female part, are such that when said male and female parts are connected, said sharp edge (112) penetrates into said catheter (106), thereby forming a fluid tight connection.

35



1  
Fig

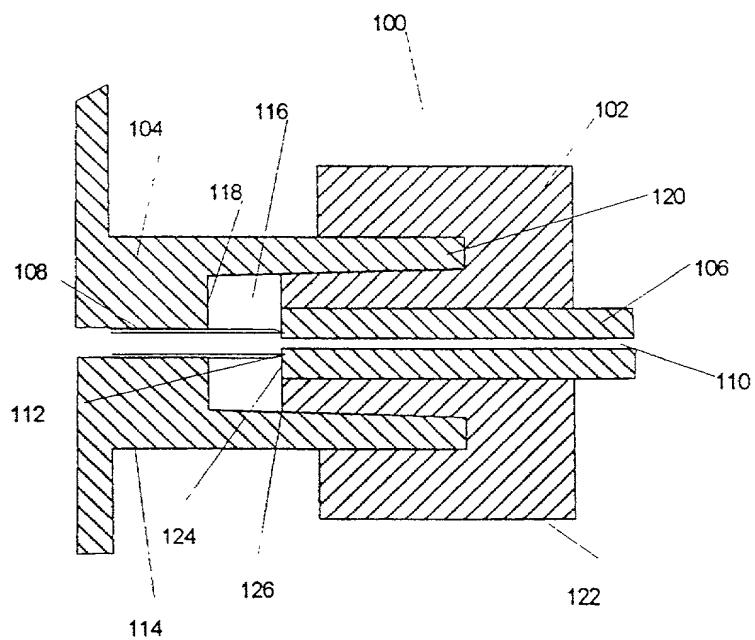


Fig 2

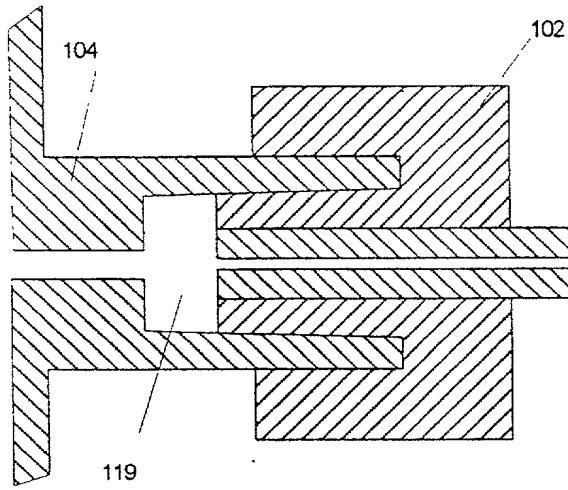


Fig 3

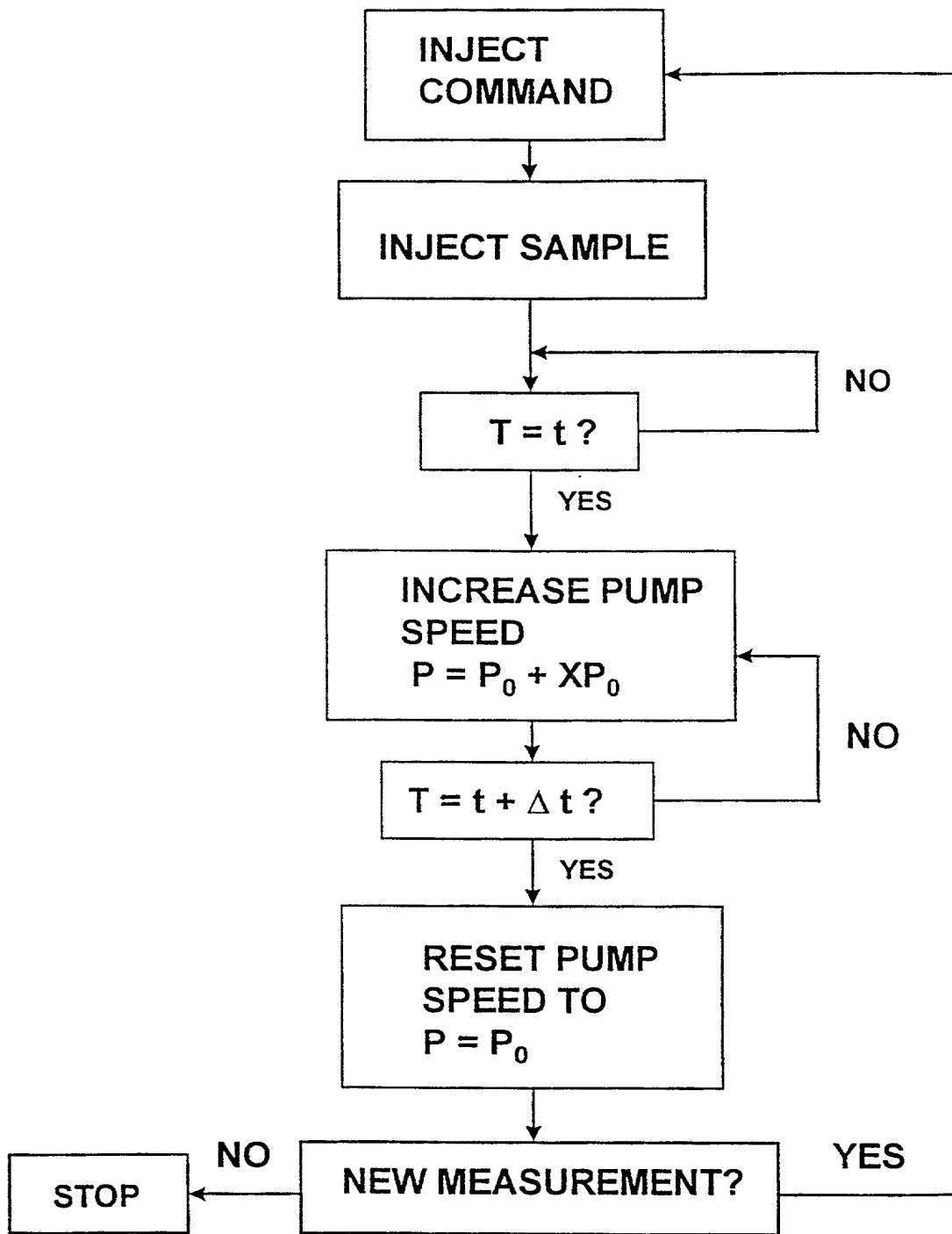


FIG. 4

PATENT APPLICATION  
DOCKET NO.: 45687-00008  
P03949US/ALi/mck

**RULES 63 AND 67 (37 C.F.R. 1.63 and 1.67)**  
**DECLARATION AND POWER OF ATTORNEY**

**FOR UTILITY/DESIGN/CIP/PCT NATIONAL APPLICATIONS**

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; and

I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: **REGENERATION OF BIOSENSORS**, the specification of which: (mark only one)

- (a) is attached hereto.
- (b) was filed on January 6, 2000 as Application Serial No. 09/462,394 and was amended on \_\_\_\_\_ (if applicable)
- (c) was filed as PCT International Application No. PCT/SE98/01352 on July 8, 1998 and was amended on \_\_\_\_\_ (if applicable).
- (d) was filed on \_\_\_\_\_ as Application Serial No. \_\_\_\_\_ and was issued a Notice of Allowance on \_\_\_\_\_.
- (e) was filed on January 6, 2000 and bearing attorney docket number 45687-00008.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims as amended by any amendment referred to above or as allowed as indicated above.

I acknowledge the duty to disclose all information known to me to be material to the patentability of this application as defined in 37 CFR § 1.56. If this is a continuation-in-part (CIP) application, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose to the Office all information known to me to be material to patentability of the application as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

I hereby claim foreign priority benefits under 35 U.S.C. § 119/365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate filed by me or my assignee disclosing the subject matter claimed in this application and having a filing date (1) before that of the application on which my priority is claimed or, (2) if no priority is claimed, before the filing date of this application:

PRIOR FOREIGN PATENTS

<u>Number</u>	<u>Country</u>	<u>Month/Day/Year Filed</u>	<u>Date first laid- open or Published</u>	<u>Date patented or Granted</u>	<u>Priority Claimed</u>
					<u>Yes</u> <u>No</u>
9702658-7	Sweden	07/09/97			X

I hereby claim the benefit under 35 U.S.C. § 120/365 of any United States application(s) listed below and PCT international applications listed above or below:

PRIOR U.S. OR PCT APPLICATIONS

<u>Application No. (series code/serial no.)</u>	<u>Month/Day/Year Filed</u>	<u>Status(pending, abandoned, patented)</u>
PCT/SE98/01352	07/08/98	pending

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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(FOR ADDITIONAL INVENTORS, check here  and add additional sheet for inventor information regarding signature, name, date, citizenship, residence and address)